

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

A61K 37/00 SH

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/00, G01N 33/53		A1	(11) International Publication Number: WO 93/00919 (43) International Publication Date: 21 January 1993 (21.01.93)
(21) International Application Number:	PCT/US92/05836	(74) Agents:	BASTIAN, Kevin, L. et al.; Townsend and Townsend, One Market Plaza, 2000 Steuart Tower, San Francisco, CA 94105 (US).
(22) International Filing Date:	13 July 1992 (13.07.92)	(81) Designated States:	JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).
(30) Priority data:	727,280 11 July 1991 (11.07.91)	US	Published <i>With international search report.</i>
(71) Applicant:	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 300 Lakeside Drive, 22nd Floor, Oakland, CA 94612-3550 (US).		<i>inf C07K 16/28 C</i>
(72) Inventors:	ROSEN, Steven ; 828 Clayton Street, San Francisco, CA 94117 (US). HUANG, Kun ; 2137 17th Avenue, San Francisco, CA 94116 (US). SINGER, Mark ; 1915 Grant Street, Berkeley, CA 94703 (US). GEOFFROY, Joyce ; 14526 So. Hoxie Avenue, Burnham, IL 60633 (US).		<i>INF C07K 15/00 H24 H</i>

*D96
D96
D96*

(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING LEUKOCYTE ADHESION TO CNS MYELIN

(57) Abstract

The present invention provides pharmaceutical compositions and methods for treating demyelinating diseases. The compositions comprise a blocking agent which inhibits LHR-mediated binding of leukocytes to myelin.

M07K 201:00

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	CN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TC	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

COMPOSITIONS AND METHODS FOR INHIBITING
LEUKOCYTE ADHESION TO CNS MYELIN

5

This invention was made with support under Grant Nos. GM23547 and AR0684 awarded by the National Institute of Health. The Government has certain rights in this invention.

10

BACKGROUND OF THE INVENTION

The present invention relates to pharmaceutical compositions and methods for treating demyelinating diseases. In particular, the invention relates to treatment using agents which inhibit leukocyte adhesion mediated by lymphocyte homing receptors (LHR).

Recent work has established that specialized cell surface receptors (termed here selectins or LEC-CAMs) on endothelial cells and various circulating cells are involved in a number of intercellular interactions. LHR (also known as gp90^{MEL}, gp100^{MEL}, gp110^{MEL}, Mel-14 antigen, Leu8 antigen, TQ1 antigen, DREG antigen, LAM-1, selectin1, LECAM-1 and LEC-CAM-1) is a selectin receptor on the surface of leukocytes and is known to be involved in the adhesive interactions of leukocytes with the endothelial lining of blood vessels. This adhesive interaction is a prerequisite for the movement of leukocytes from the blood to tissue sites where immune reactions and inflammatory reactions occur. LHR is also important for lymphocyte homing from the blood into secondary lymphoid organs.

30

All selectins share certain structural features, including a lectin-like region which recognizes specific carbohydrate-containing ligands. For a review of selectin receptors see, Springer, Nature, 346:425 (1989), which is incorporated herein by reference. Other selectin receptors are found on endothelial cells and platelets. Endothelial leukocyte adhesion molecule-1 (ELAM-1) is present on endothelial cells and is involved in the recognition of various circulating cells by the endothelium. Granule membrane

35

protein-140 (GMP-140) is present on the surface of platelets and endothelial cells, where it mediates platelet-leukocyte and endothelium-leukocyte interactions.

There is currently an interest in developing highly specific competitive inhibitors of selectin-mediated cellular adhesion. Such inhibitors are useful in therapeutic regimens to treat various selectin-mediated disease responses. In particular, little is known about the role selectins might play in responses other than inflammation and lymphocyte homing.
10 Identification of other interactions involving selectin receptors will open new paths to therapy for other disease processes.

SUMMARY OF THE INVENTION

15 The present invention concerns pharmaceutical compositions and methods which are useful for treating and diagnosing demyelinating diseases. The claimed pharmaceutical compositions comprise a pharmaceutically acceptable carrier and a blocking agent which inhibits LHR-mediated binding of 20 leukocytes to myelin. The claimed methods use these compositions for treating and diagnosing demyelinating diseases, such as multiple sclerosis.

The blocking agents of the present invention function by selectively binding either LHR or the recognition 25 determinant on myelin. Those blocking agents which selectively bind LHR are typically carbohydrates or compounds which comprise a carbohydrate moiety which selectively binds LHR. Carbohydrates of the present invention include mannose-6-phosphate, fructose-1-phosphate or fragments of fucoidin or the 30 phosphomannan monoester core from Hansenula hostii (PPME). Compounds which comprise an LHR-binding moiety include glycolipids, such as sulfatide, and glycoproteins, such as endothelial cell surface glycoproteins. The glycoproteins are preferably an extracellular region of Sgp⁵⁰ or Sgp⁹⁰. The 35 blocking agent may also be an immunoglobulin which reacts with LHR, such as TQ1 and LAM 1.4.

Blocking agents which selectively bind the recognition determinant on myelin are typically isolated LHR,

which may be in soluble form or embedded in a lipid membrane. Soluble forms of LHR preferably comprise an LHR component and an immunoglobulin component.

5

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As discussed above, LHR is known to be involved in a number of physiological responses. For instance, the trafficking of lymphocytes from the blood into secondary lymphoid organs, such as lymph nodes and gut-associated Peyer's patches, is known to be initiated by an adhesive interaction between specialized endothelial cells of high endothelial venules (HEV) and LHRs on lymphocytes. Berg et al., Immunol. Rev. 108:5-18 (1989); Duijvestijn and Hamann, Immunol. Today 10:23-28 (1989); Woodruff et al., Ann. Rev. Immunol. 5:201-222 (1987); Yednock and Rosen, Adv. Immunol. 54:313-378 (1989); Stoolman, Cell 56:907-910 (1989); Gallatin et al., Cell 44:673-680 (1986); Rosen, Curr. Opin. Cell. Biol. 1:913-919 (1989), all of which are incorporated herein by reference. In addition, LHR on neutrophils, monocytes, and eosinophils mediates the early interaction of these cells with endothelium of blood vessels at sites of inflammation (Gallatin, et al., Nature 303:30 (1983) and Lewinsohn, et al., J. Immunol. 138:4313 (1987), which are incorporated herein by reference).

The lectin domain on LHR for lymph nodes in humans and mice was initially inferred based upon the ability of specific phosphorylated monosaccharides, such as mannose-6-phosphate (M6P), and specific polysaccharides to prevent lymphocyte attachment to HEV (Stoolman and Rosen, J. Cell Biol. 96:722-729 (1983); Stoolman et al., J. Cell Biol. 99:1535-1540 (1984); Yednock et al., J. Cell Biol. 104:713-723 (1987); Stoolman et al., Blood 70:1842-1850 (1987); Stoolman and Ebling, J. Clin. Invest. 84:1196-1205 (1989) all of which are incorporated herein by reference). Notable among the active polysaccharides are PPME (a phosphate-rich mannan core) and fucoidin (a sulfated, fucose-rich polymer). This carbohydrate-binding activity depends on the presence of calcium, which is also required for the attachment of lymphocytes to HEV.

From the lectin nature of LHR, the ligands on lymph node HEV are presumed to bear a carbohydrate-based recognition determinant. Early studies demonstrated that the adhesive sites on peripheral lymph node HEV are periodate sensitive
5 (Rosen et al., Science 228:1005-1007 (1985) which is incorporated herein by reference), indicating a requirement for carbohydrate. Subsequently, it was demonstrated that sialidase treatment of HEV, in vitro or in vivo, selectively eliminates lymphocyte attachment to peripheral lymph node HEV but has no
10 effect on the binding to Peyer's patch HEV (Rosen et al., J. Immunol. 142:1895-1902 (1989) which is incorporated herein by reference). In addition, exposure of peripheral lymph node tissue sections to Limax flavus agglutinin, a sialic acid-specific lectin, prevents lymphocyte attachment to HEV (True et
15 al., J. Cell Biol. 111:2757-2764 (1990) which is incorporated herein by reference).

The structure and function of selectin receptors has been elucidated by cloning and expression of full length cDNA encoding each of the receptors (see, e.g., Bevilacqua et al.,
20 Science, 243:1160 (1989) (ELAM-1), Geng et al., 343:757-760 (1990) (GMP 140), and Lasky et al., Cell 56:1045-1055 (1989) (LHR) which are incorporated herein by reference). The extracellular portion of selectins can be divided into three segments based on homologies to previously described proteins.
25 The N-terminal region (about 120 amino acids) is related to the C-type mammalian lectin protein family as described by Drickamer, J. Biol. Chem., 263: 9557-9560 (1988) (which is incorporated herein by reference) that includes low affinity IgE receptor CD23. A polypeptide segment follows, which has a
30 sequence that is related to proteins containing the epidermal growth factor (EGF) motif. Lastly, after the EGF domain are one or more tandem repetitive motifs of about 60 amino acids each, related to those found in a family of complement regulatory proteins.

35 A basis for the present invention is the discovery that LHR plays a role in mediating binding of leukocytes to myelinated regions of the central nervous system (CNS). The myelin sheath is a layer comprised primarily of lipid and

protein which surrounds the axons of neurons in the central and peripheral nervous systems. The sheath acts as an electrical insulator by preventing the transport of ions across the neuron membrane. In the CNS, the myelin sheath is formed from the 5 plasma membrane of oligodendrocytes which envelop the axon.

A number of neurological disorders are the result of demyelination of axons in the CNS. Demyelinating diseases typically involve patchy destruction of the myelin sheath to form cleared regions of the axon referred to as plaques.

10 Typically, the disease is accompanied by an inflammatory response, as well. Demyelinating diseases include multiple sclerosis (MS), acute disseminated encephalomyelitis, acute necrotizing hemorrhagic encephalomyelitis, and HIV associated myopathy. MS is the most common of the demyelinating 15 diseases and is generally thought to involve autoimmunity, perhaps induced by viral infection. For a brief review of MS and other demyelinating diseases, see, Antel et al., in Harrison's Principles of Internal Medicine, 12th ed., Wilson et al. eds, (McGraw Hill, New York), which is incorporated herein 20 by reference.

The evidence provided here shows that LHR plays a role in the pathogenesis of demyelinating diseases such as MS. LHR is a targeting molecule involved in the selective destruction of the myelin sheaths of CNS neurons. Because LHR 25 is expressed by most lymphocytes, regardless of antigen specificity, non-immunogenic responses must be involved in the etiology of these diseases and other conditions such as traumatic injury to the spinal cord. LHR thus mediates association of other leukocytes, such as monocytes, neutrophils, basophils, and eosinophils to myelinated sheaths. These cells may gain entry into the brain or other parts of the CNS (normally a privileged site devoid of leukocytes) through, for example, traumatic injury to the spinal cord. Selective damage then results from a number of mechanisms such as cell-mediated cytotoxicity or from the local release of cytokines, 30 proteases, or free-radicals.

With the discovery of this new role for LHR, agents known to block LHR-mediated adhesion can be used in treating

demyelinating disease. The blocking agents of the present invention function either by selectively binding LHR (*i.e.*, substituting for the recognition determinant on myelin) or by selectively binding the recognition determinant on myelin (i.e., substituting for LHR on the leukocyte). Assays which identify compounds able to block LHR-mediated binding can be used to identify a wide range of compounds useful in the present invention. Copending application U.S.S.N. 07/695,805, which is incorporated herein by reference, discloses a number of assays useful in identifying such compounds.

Blocking agents of the present invention selectively bind either LHR or the recognition determinant on myelin. Selective binding as used herein refers to specific recognition by one molecule (typically referred to as a receptor) of another molecule (typically referred to as a ligand) by the spatial or polar organization of a recognition determinant on the second molecule. Selective binding is said to occur when the binding affinity between the molecules is sufficiently high. Binding affinity is typically represented by the affinity constant (K_a) for equilibrium concentrations of associated and disassociated configurations, *i.e.*, $K_a = [R-L]/[R][L]$ where [R], [L], and [R-L] are the concentrations at equilibrium of the receptor (R), ligand (L) and receptor-ligand complex (R-L), respectively. Under physiological conditions, the affinity constant of a blocking agent of the present invention is typically about 10^4 to about 10^8 liters/mole, and preferably about 10^8 liters/mole or more. One of skill will recognize, however, that binding affinity between two molecules will be influenced by a number of factors such as temperature, pH, ionic strength, and the like.

A number of compounds which selectively bind LHR are useful as blocking agents in the present invention. Thus these compounds act as antagonists to the myelin recognition determinant. As used herein, the recognition determinant is the minimal structure on myelin which selectively binds LHR. Antagonists are compounds which reverse the physiological effect of a ligand or exclude binding of the ligand to a receptor. An antagonist competes directly or indirectly with

the ligand for the receptor binding site and, thus, reduces the proportion of ligand molecules bound to the receptor.

Typically, an antagonist is the topographical equivalent of the natural ligand and will compete directly with the ligand for

5 the binding site on the selectin. Such a compound is referred to here as a "mimetic." A ligand mimetic is a molecule that conformationally and functionally serves as substitute for the natural ligand recognized by a selectin receptor.

Alternatively, if the ligand and the blocking agent can bind
10 the receptor simultaneously, the compound may act non-
competitively. A non-competitive inhibitor acts by decreasing
or inhibiting the subsequent physiological effects of receptor-
ligand interactions rather than by diminishing the proportion
of ligand molecules bound to the receptor.

15 A blocking agent of the present invention which
selectively binds LHR is typically a synthetic or naturally-
produced biomolecule, such as a carbohydrate (e.g.,
oligosaccharide) or a glycoconjugate comprising a structure
specifically recognized by LHR. Biomolecules as defined here
20 include but are not limited to biologically significant
molecules such as amino acids (and their mimetics),
oligopeptides, proteins (e.g., glycoproteins and protein
hormones), fatty acids, lipids (e.g., glycolipids,
phospholipids, sphingolipids and gangliosides, such as GM₁, GM₂,
25 and the like), steroid hormones, oligosaccharides, polysaccharides,
and nucleic acids (e.g., deoxyribonucleic acids and ribonucleic
acids). The blocking agent is preferably a relatively small
molecule with a molecular weight less than about 10 kD,
preferably less than about 5 kD.

30 A number of carbohydrate-containing compounds which
selectively bind LHR are conveniently used as the blocking
agent in the present invention. For instance, phosphorylated
monosaccharides, such as mannose-6-phosphate and fructose-1-
phosphate inhibit lymphocyte attachment to HEV in in vitro
35 cellular assays. Polysaccharides and glycolipids, have also
been shown to inhibit in vitro binding of these cells (Stoolman
et al., Blood 70:1842-1850 (1987); Yednock et al., J. Cell
Biol. 104:713-723 (1987); and Yednock et al., J. Cell Biol.,

104:725-731 (1987), and Imai et al., J. Cell Biol. 111:1225-1232 (1990) which are incorporated herein by reference.

The carbohydrate-containing compounds of the present invention are typically phosphorylated, sulfated, sialylated, and/or fucosylated. One of skill will readily recognize that, using standard techniques (e.g., enzymatic or chemical synthesis) oligosaccharides capable of selectively binding LHR can be prepared. These compounds can then be screened using standard methods (e.g., those in the example section, below) to determine the ability of the carbohydrates to inhibit binding to myelinarted sheaths.

Phosphorylated polysaccharides of the present invention include the phosphomannan monoester core from Hansenula hostii (PPME). Sulfated polysaccharides include fucoidin, egg jelly fucan and dextran sulfate. Sulfated glycolipids of the invention include sulfatide. Sialylated glycolipids (e.g., gangliosides) include GM₁, GM₂, GD_{1a} and the like. Typically, fragments which retain LHR-binding ability are used in the present invention. One of skill will readily recognize methods for preparing and assaying the appropriate fragments of these compounds. The fragments will typically have a molecular weight of less than about 10 kD, preferably less than about 5 kD.

Lymph node endothelial cell surface sialylated, sulfated glycoproteins which comprise oligosaccharide biological ligands specifically recognized by LHR can also be used in the present invention. As demonstrated in copending application U.S.S.N. 07/695,805, two such glycoproteins, Sgp⁵⁰ and Sgp⁹⁰, have been identified (see, also, Imai et al., J. Cell Biol. 113:1213-1221 (1991), which is incorporated herein by reference). Having identified ligand-bearing glycoproteins, one of skill will recognize that a number of modifications of the glycoproteins that do not significantly alter the LHR binding activity are possible. Such modifications include enzymatic or chemical treatment of the proteins to produce fragments that comprise the carbohydrate ligand recognized by LHR. For instance, fragments of the proteins can be obtained

by treatment with an appropriate protease such as trypsin, pronase, papain, pepsin and the like.

The fragments of the present invention typically comprise at least a portion of the glycoprotein extracellular region (*i.e.*, that portion which comprises a carbohydrate ligand recognized by LHR and which is outside the transmembrane and intracellular regions). Because the extracellular region substantially lacks the hydrophobic transmembrane region, it is typically water soluble. The extracellular region, however, may also contain sequences from the transmembrane region (less than about 10 amino acids), so long as solubility is not substantially affected.

As used here, a compound comprising the extracellular region includes any compound in which at least a portion of the extracellular region is conjugated to a second moiety. The term also embraces the isolated extracellular region and the isolated full length glycoprotein, or fragment thereof. An isolated compound comprising the extracellular region includes such a compound (*e.g.*, a full length glycoprotein) in other than its native state, that is, not associated with an endothelial cell. For instance, the compound may be recombinantly produced, solubilized from the appropriate cell, or associated with a synthetic lipid membrane, for example, a liposome. Methods for preparing liposomes are well known in the art, see, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028, incorporated herein by reference.

Analysis of the sulfated glycoproteins of the present invention has revealed that the oligosaccharide moieties recognized by LHR are O-linked. Thus, they can be cleaved from the protein backbones by beta elimination and borohydride reduction according to standard techniques (see, e.g., Fukuda, Meth. Enzymol. 179:17-29 (1989), which is incorporated herein by reference). Once cleaved, oligosaccharides can be conjugated to any number of other compounds. For instance, they can be conjugated to a biomolecule using standard techniques. Neoglycoproteins, neoglycolipids or cluster glycosides can be prepared based on the carbohydrate chains of

the glycoproteins using methods well known in the art (see, e.g., Stowell et al., Adv. Carb. Chem and Biochem. 37:225-281 (1980), Childs et al. Biochem. J., 262:131-138 (1989), and Lee et al., Glycoconjugate J. 4:317-328 (1987), which are 5 incorporated herein by reference).

The sulfated glycoproteins can be isolated using a number of techniques. For instance, soluble LHR can be used to identify the glycoproteins in a preparation of proteins isolated from endothelial cells. The glycoproteins can be used 10 as they are isolated or they can be modified according to techniques well known in the art. For instance, the extracellular region can be conjugated to a variety of other compounds (e.g., immunoglobulin constant regions) to confer any 15 number of desired characteristics, such as improved solubility, serum half-life and the like. For a description of methods for making novel derivatives of cell surface proteins which comprise immunoglobulin constant regions see, EP Patent Application No. 88309194.4, which is incorporated herein by reference.

Blocking agents which selectively bind LHR can be easily prepared from commonly available starting materials. Biomolecules can be isolated from any natural source, such as animal, plant, fungal, or prokaryotic cells in accordance with standard procedures. For instance, PPME is purified from crude 25 yeast mannan by the method of Slodki et al., Biochim. Biophys. Acta, 304:449-456 (1973), which is incorporated herein by reference. Briefly, the phosphomannan is acid hydrolyzed. After neutralization, the phosphomannan core is precipitated and rehydrated in water. Contaminating protein is removed by 30 water:chloroform:butanol extraction. Alternatively, many polysaccharides (such as fucoidin) and glycolipids can be purchased from chemical supply companies, such as Sigma Chemical Co. (St. Louis, MO) and Aldrich Chemical Co. (Milwaukee, WI).

Many blocking agents are synthetically produced using standard methods. See, e.g., Khadem, Carbohydrate Chemistry (Academic Press, San Diego, CA, 1988), which is incorporated herein by reference, for synthesis of carbohydrates. Methods

for synthesizing polypeptides of defined composition are well known in the art (see, Atherton et al. Solid Phase Peptide Synthesis (IRL Press, Oxford, 1989) which is incorporated herein by reference).

5 The blocking agents of the present invention can also be agents which selectively bind the recognition determinant on the myelin sheath. For instance, isolated LHR can be used to block adhesion. The term "isolated LHR" as used herein refers to an LHR molecule, or fragment thereof, in other than its
10 native state, for example, not associated with the cell which normally expresses it. As discussed above, cDNA encoding human LHR has been isolated. Thus, LHR, or fragments thereof, can be recombinantly produced using standard methods well known to those skilled in the art. For a review of standard molecular
15 biological techniques see Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d Ed. (Cold Spring Harbor Press, N.Y., 1989), which is incorporated herein by reference. In addition, using standard recombinant DNA techniques, mutations can be induced to obtain proteins with altered amino acid sequences.
20 Typically, substitutions, deletions or additions are introduced which provide desired characteristics. For instance, increased solubility can be achieved by elimination of the hydrophobic transmembrane region of the protein. In addition, soluble chimeric receptors comprising the constant region of an
25 immunoglobulin molecule, termed here LHR-IgG, can also be produced (Watson et al., J. Cell Biol. 110:2221-2229 (1990), and Watson et al., Nature 349:164-167 (1991) which are incorporated herein by reference). LHR, or fragments thereof, can also be associated with synthetic lipid membranes as
30 described for the glycoproteins, above.

In addition to treatment of demyelinating diseases, isolated LHR (e.g., LHR-IgG) can be used for diagnosis or monitoring the state of the disease. For instance, by measuring the increase or decrease in the number of
35 demyelinated plaques it is possible to determine whether a particular therapeutic regimen aimed at ameliorating the disease is effective.

For in vivo diagnostic imaging, radioisotopes are typically used in accordance with well known techniques. The radioisotopes may be bound to LHR either directly or indirectly using intermediate functional groups well known to those skilled in the art. For instance, chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules have been used to bind proteins to metallic ion radioisotopes.

LHR can also be labeled with a paramagnetic isotope for purposes of in vivo diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be used. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes are used for MRI.

Immunoglobulins which recognize either LHR or its ligands can also be used to block LHR-myelin interactions. For instance, TQ1 and LAM 1.4 are monoclonal antibodies that react with human LHR and effectively interfere with lymphocyte attachment to HEV (Tedder, et al. J. Immunol., 144:532 (1990), which is incorporated herein by reference). Other monoclonal antibodies known to bind human LHR include Leu-8 (Cameriai et al., Nature 342:78-82 (1989), which is incorporated herein by reference) and the DREG antibodies (Kishimoto et al., PNAS 87:2244-2248 (1990), which is incorporated herein by reference). Leu-8 is commercially available through Becton Dickinson.

The multitude of techniques available to those skilled in the art for production and manipulation of various immunoglobulin molecules can thus be readily applied to inhibit adhesion of leukocytes to myelin sheaths. As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Immunoglobulins may exist in a variety of forms besides antibodies, including for example, Fv, Fab, and F(ab)₂, as well

as in single chains (e.g., Huston et al., Proc. Nat. Acad. Sci. U.S.A. 85:5879-5883 (1988) and Bird et al., Science 242:423-426 (1988), and Hunkapiller and Hood, Nature 323:15-16 (1986), which are incorporated herein by reference). For a general 5 review of immunoglobulin structure and function see, Fundamental Immunology, 2d Ed., W.E. Paul ed., Ravens Press, N.Y., (1989) which is incorporated herein by reference.

Antibodies which bind either LHR or its ligands may be produced by a variety of means. The production of non-human 10 monoclonal antibodies, e.g., murine, lagomorpha, equine, etc., is well known and may be accomplished by, for example, immunizing the animal with a preparation containing LHR or the appropriate ligand. Antibody-producing cells obtained from the immunized animals are immortalized and screened, or screened 15 first for the production of antibody which inhibits the interaction of the myelin sheath with LHR and then immortalized. For a discussion of general procedures of monoclonal antibody production see Harlow and Lane, Antibodies, A Laboratory Manual Cold Spring Harbor Publications, N.Y. 20 (1988), which is incorporated herein by reference.

The generation of human monoclonal antibodies to a human antigen (in the case of LHR isolated from human tissue) may be difficult with conventional techniques. Thus, it may be desirable to transfer the antigen binding regions of the non- 25 human antibodies, e.g., the F(ab'), or hypervariable regions, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules. Such methods are generally known in the art and are described in, for example, U.S. 4,816,397, EP publications 173,494 and 239,400, which are incorporated herein 30 by reference. Alternatively, one may isolate DNA sequences which encode a human monoclonal antibody or portions thereof that specifically bind to the human LHR by screening a DNA library from human B cells according to the general protocol 35 outlined by Huse et al., Science 246:1275-1281 (1989), incorporated herein by reference, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

Immunoglobulins which inhibit the binding of LHR to myelin may also be useful in the generation of anti-idiotypic immunoglobulins. Anti-idiotype immunoglobulins may be produced by, for example, immunization of an animal with the primary immunoglobulin. In the case of immunoglobulins to LHR, those anti-idiotype immunoglobulins whose binding to the primary immunoglobulin is inhibited by LHR are selected. Since both the anti-idiotypic immunoglobulin and the receptor bind the primary immunoglobulin, the anti-idiotypic immunoglobulin may represent the "internal image" of an epitope and thus may substitute for the receptor and be used, for example, as an immunogenic reagent.

The present invention specifically provides pharmaceutical compositions which are useful in treating or diagnosing the demyelinating diseases discussed above. The pharmaceutical compositions are comprised of the blocking agents together with pharmaceutically acceptable carriers. The pharmaceutical compositions can be prepared according to standard methods (see Remington's Pharmaceutical Sciences, Mack Publishing Co., Philadelphia, PA, 19th ed. (1985) which is incorporated herein by reference). The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see, Langer, Science 249:1527-1533 (1990), which is incorporated herein by reference.

For pharmaceutical compositions which comprise the blocking agents of the present invention, the dose will vary according to, e.g., the particular agent, the manner of administration, the particular disease being treated and its severity, the overall health and condition of the patient, and the judgment of the prescribing physician. Total dosages typically range between about 1 and about 10 mg/kg, preferably about 2 to about 7 mg/kg. Since the present invention provides evidence of a mechanism for demyelinating diseases, maximization of dosage levels for inhibition of LHR-mediated adhesion can now be achieved by one of ordinary skill in the art. "Substantial inhibition" of binding for purposes of the

present invention is preferably at least about 70% inhibition, preferably 80% to 90% and most preferably 95%, or more.

The pharmaceutical compositions are intended for parenteral, topical, oral or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, and capsules.

Preferably, the pharmaceutical compositions are administered directly into the cerebral spinal fluid of the CNS by intrathecal injection. Thus, this invention provides compositions for which comprise a solution of the complex dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, phosphate buffered saline, 0.4% saline, and the like. These compositions may be sterilized by conventional, well known sterilization techniques. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of the complex can vary widely, i.e., from less than about 0.05%, usually at or at least about 1% to as much as 10 to 30% by weight and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium

carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of 5 active ingredient.

For aerosol administration, the complexes are preferably supplied in finely divided form along with a surfactant and propellant. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant.

10 Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride such as, for 15 example, ethylene glycol, glycerol, erythritol, arabitol, mannitol, sorbitol, the hexitol anhydrides derived from sorbitol, and the polyoxyethylene and polyoxypropylene derivatives of these esters. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may 20 constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. Liquefied propellants are typically gases at ambient conditions, and are condensed under pressure. Among suitable liquefied propellants are the lower alkanes containing 25 up to 5 carbons, such as butane and propane; and preferably fluorinated or fluorochlorinated alkanes. Mixtures of the above may also be employed. In producing the aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds 30 and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve.

The compositions containing the blocking agents can be administered for therapeutic, prophylactic, or diagnostic applications. In therapeutic applications, compositions 35 containing the agents, or a cocktail thereof, are administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An

amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient.

5 In prophylactic applications, compositions containing the blocking agents, or a cocktail thereof, are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend
10 on the patient's state of health and weight.

In diagnostic applications, compositions containing the blocking agents, or a cocktail thereof, are administered to a patient suspected of having a demyelinating disease state to determine the presence of plaques associated with the disease.
15 Alternatively, the efficacy of a particular treatment can be monitored. An amount sufficient to accomplish this is defined to be a "diagnostically effective dose." In this use, the precise amounts will depend upon the patient's state of health and the like.

20 Kits can also be supplied for therapeutic or diagnostic uses. Thus, the subject composition of the present invention may be provided, usually in a lyophilized form in a container. The agents, which may be conjugated to a label or unconjugated, are included in the kits with buffers, such as
25 Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% by weight based on the amount of blocking agent and usually present in total amount of at least
30 about 0.001% by weight based again on the protein concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% by weight of the total composition. Where an antibody is
35 employed, this will usually be present in a separate vial. The antibody is typically conjugated to a label and formulated according to techniques well known in the art.

The following examples are offered by way of illustration, not by way of limitation.

Example 1

5 Binding of LHR-IgG to Myelinated Regions of CNS

This example describes immunohistochemical staining of CNS using LHR-IgG. The method was as modified from Watson, et al., J. Cell Biol., supra. Briefly, cryostat-cut tissue sections (10 µm) were fixed with 0.5% paraformaldehyde in 0.1 M sodium cacodylate (pH 7.3) for 20 minutes on ice, followed by immersion in 100% methanol with 0.3% H₂O₂ for 20 minutes on ice. The sections were washed in Dulbecco's PBS (PBS) and incubated for 80 minutes on ice with 30 µg/ml LHR-IgG in PBS with 5% normal horse serum and 5% normal rat serum. They were then washed and incubated with biotinylated-goat anti-human IgG (Zymed Laboratories, South San Francisco, CA) in PBS containing 5% normal mouse serum for 30 minutes at room temperature followed by an ABC Elite reagent (Vector Labs, Burlingame, CA), and then AEC peroxidase substrate (Biomedica, Foster City, CA). Finally, the sections were counterstained with aqueous hematoxylin (Biomedica). The addition of 5% normal horse serum and 5% normal rat serum was necessary to eliminate nonspecific binding of the LHR-IgG to myelin.

The above method revealed specific staining of the myelin-rich white matter tracts of both cerebellum and spinal cord. In contrast, regions having few or no myelinated fibers such as the granular and molecular layers of the cerebellum and the grey matter of the spinal cord were negative. The addition of EGTA greatly diminished specific staining, in agreement with the known calcium-dependent binding of LHR (see, Yednock et al. J. Cell Biol., 104:713, supra). Staining of myelin by LHR-IgG was restricted to CNS myelin. Myelin of the peripheral nervous system was not stained. This result further supports the role of LHR in mediated demyelinating diseases restricted to the CNS.

35

Example 2

Effects of Antibodies, EGTA, and Carbohydrates on

Lymphocyte Binding to Cerebellar Myelin

This example demonstrates that both antibodies directed to LHR and EGTA inhibit lymphocyte binding to cerebellar myelin. The integrins, VLA-4 ($\alpha_4\beta_1$), LPAM-1 ($\alpha_4\beta_p$) and LFA-1 ($\alpha_1\beta_2$) are known to participate in a variety of lymphocyte adhesive interactions. Therefore, function-blocking antibodies against α_4 , β_1 , and β_2 were also used in the assay to test the role of these receptors in adhesion to myelin.

Lymphocyte binding assays on serial sections of mouse cerebellum and spinal cord were based on the method of Stamper and Woodruff J. Exp. Meth 144:828 (1976), which is incorporated herein by reference. Briefly, mouse mesenteric lymph node lymphocytes (10^6 cells in 100 μ l in RPMI 1640 supplemented with 5% FBS and 12.5 mM Hepes) were incubated on paraformaldehyde-fixed tissue sections on ice in a gyratory shaker for 30 minutes at 80 rpm. The sections were then fixed in 2.5% glutaraldehyde and stained with toluidine blue. The appropriate antibodies were added during the 30 minute incubation period. The number of bound cells per unit area (UA) was then determined microscopically. The unit area was defined with the aid of an ocular reticle or as a defined myelin strip present on a series of contiguous sections of the cerebellum.

The results of these experiments are presented in Table 2. The number of cells bound per UA (\pm SEM) was derived from 3-5 replicate sections. The cells used were as follows: mouse mesenteric lymph node lymphocytes (MNL), Jurkat JS9-78 (a cloned T cell line with high expression of LHR) and human peripheral blood mononuclear leukocytes (PBL) isolated from peripheral blood of healthy volunteers using Mono-Poly Resolving Medium (Flow Laboratories, Inc., McLean, VA).

The antibodies were used at the following concentrations: MEL-14, 5 μ g/ml; PolyMEL serum (from a rabbit immunized with purified mouse LHR) 1:10, LAM1.4, 1:100; anti- β_2 ascites (Telios Pharmaceuticals, Inc., San Diego, CA), 1:50; anti-VLA4 (HP2/1, AMAC, Inc., Westbrook, ME), 4 μ g/ml (This antibody inhibited rat lymphocyte binding to Peyer's patch by

more than 70% in an in vitro binding assay); and TQ1 (Coulter Lab, Hialeah, FL), 10 μ g/ml. EGTA was used at 10 mM.

Table 1

5	Exper-	Cerebellum	Cells	Reagent	Cells	% Change
	iment	source	for	added	bound/UA	Compared to
			binding			Control
10	1	mouse	MNL	none	140.3 (\pm 12.2)	
				EGTA	11.7 (\pm 1.8)	-91.7
	2	human	MNL	none	96.7 (\pm 5.5)	
				MEL-14	38.5 (\pm 8.5)	-60.2
				PolyMEL	56.0 (\pm 10.7)	-42.1
15	3	mouse	Jurkat	none	296.7 (\pm 44.9)	
				TQ1	7.3 (3.2)	-97.5
				PolyMEL	6.3 (\pm 3.2)	97.9
	4	mouse	Jurkat	none	82.0 (\pm 27.3)	
				TQ1	5.7 (\pm 2.4)	-93.1
20				LAM1.4	15.0 (\pm 6.5)	-81.7
	5	human	Jurkat	none	203.5 (\pm 12.0)	
				TQ1	8.3 (\pm 3.4)	-95.9
				anti- β 2	189.3 (\pm 5.3)	-7.0
				anti-VLA4	192.8 (\pm 5.8)	-5.3
25				EGTA	3.8 (\pm 1.5)	-98.1
	6	mouse	human			
			PBL	none	267.3 (\pm 67.6)	
				TQ1	5.0 (\pm 1.5)	-98.1
				anti- β 2	308.0 (\pm 19.1)	+15.2
30				anti-VLA4	283.0 (\pm 50.1)	+6.0
				EGTA	12.0 (\pm 4.0)	-95.5

Various carbohydrates were also tested for inhibition of human lymphocyte binding to myelinated regions in the above assays. Carbohydrates tested were PPME, mannose-6-phosphate, and fructose-1-phosphate. Complete inhibition of binding was seen by PPME at 10 μ g/ml and by the monosaccharides at 10mM . The results above show that both monoclonal antibodies that react with human LHR and carbohydrates block binding of both T

cells and human PBLs to the same extent as EGTA. In addition, antibodies that react with functional regions of integrin receptors were without effect.

5

Example 3

Effects of phorbol ester on Lymphocyte Binding
to Cerebellar Myelin

This example demonstrates that phorbol ester (PMA) inhibits Jurkat cell binding to cerebellar myelin. PMA treatment of human lymphocytes is known to cause a rapid and almost complete shedding of LHR from the cell surface (Tedder et al., *supra*). Jurkat cells were incubated with PMA at 100 ng/ml for 30 minutes at 37°C or left untreated under the same conditions. After washing, they were tested in the binding assay as described above. Numbers represent cells bound per unit area and % inhibition (\pm SEM). EGTA was used at 10 mM. The results presented in Table 2 below, show that PMA-induced shedding of LHR inhibited T cell binding to both human and mouse myelin sheaths. FACS analysis confirmed that PMA treatment decreased LHR expression by 87% relative to untreated cells.

Table 2

	Cerebellum source	Treatment of cells	EGTA	Cells bound/UA	% inhibition
30	mouse	none	-	130.0 (\pm 19.4)	
			+	12.0 (\pm 3.7)	90.8 (\pm 2.9)
	human	PMA	-	10.0 (\pm 3.5)	92.3 (\pm 2.7)
			+	4.5 (\pm 1.3)	96.5 (\pm 1.0)
35	mouse	none	-	117.0 (\pm 4.1)	
			+	2.5 (\pm 1.0)	97.9 (\pm 0.8)
	human	PMA	-	4.5 (\pm 0.6)	96.2 (\pm 0.6)
			+	2.8 (\pm 0.5)	97.6 (\pm 0.4)

These results provide further evidence that LHR is involved in the adhesion of leukocytes to myelin. Shedding of LHR by treatment with PMA almost completely inhibited binding to myelin.

5

The examples above demonstrate the ability of the agents of the present invention to effectively block LHR-mediated leukocyte adhesion to myelin. For the purposes of clarity and understanding, the invention has been described in 10 these examples and the above disclosure in some detail. It will be apparent, however, that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating a demyelinating disease in a patient, the method comprising administering to the patient a therapeutically effective dose of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a blocking agent which inhibits LHR-mediated binding of leukocytes to myelin, the blocking agent being present in an amount to substantially inhibit LHR-mediated adhesion.

10 2. A method of claim 1, wherein the blocking agent selectively binds LHR.

3. A method of claim 2, wherein the blocking agent is a carbohydrate.

15 4. A method of claim 3, wherein the carbohydrate is mannose-6-phosphate, fructose-1-phosphate.

20 5. A method of claim 3, wherein the carbohydrate is a fragment of fucoidin or PPME.

25 6. A method of claim 2, wherein the blocking agent comprises a carbohydrate moiety which selectively binds LHR, the carbohydrate having at least one of the following a sialic acid residue, fucose residue, or a sulfate group.

7. A method of claim 2, wherein the blocking agent is an oligosaccharide.

30 8. A method of claim 2, wherein the blocking agent is a glycoprotein or glycolipid.

35 9. A method of claim 2, wherein the blocking agent comprises an extracellular region of an endothelial cell surface glycoprotein.

10. A method of claim 9, wherein the endothelial cell surface glycoprotein is Sgp⁵⁰ or Sgp⁹⁰.

11. A method of claim 2, wherein the blocking agent is an immunoglobulin.

12. A method of claim 1, wherein the blocking agent 5 selectively binds a recognition determinant on myelin.

13. A method of claim 12, wherein the blocking agent is an isolated LHR.

10 14. A method of claim 13, wherein the blocking agent comprises an LHR component and an immunoglobulin component.

15 15. A method of claim 13, wherein the isolated LHR is embedded in a lipid membrane.

16. A method of claim 1, wherein the demyelinating disease is multiple sclerosis.

20 17. A pharmaceutical composition for treating a demyelinating disease, the composition comprising a pharmaceutically acceptable carrier and a blocking agent which inhibits LHR-mediated binding of leukocytes to myelin, the blocking agent being present in an amount sufficient to effectively treat the demyelinating disease.

25 18. A composition of claim 17, wherein the blocking agent selectively binds LHR.

30 19. A composition of claim 18, wherein the blocking agent is mannose-6-phosphate, fructose-1-phosphate.

20. A composition of claim 18, wherein the carbohydrate is a fragment of fucoidin or PPME.

35 21. A composition of claim 18, wherein the blocking agent comprises an extracellular region of an endothelial cell surface glycoprotein.

22. A composition of claim 18, wherein the endothelial cell surface glycoprotein is Sgp⁵⁰ or Sgp⁹⁰.

23. A composition of claim 17, wherein the
5 demyelinating disease is multiple sclerosis.

24. A method of blocking LHR-mediated adhesion of leukocytes to myelin in a patient, the method comprising administering to the patient a therapeutically effective dose 10 of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a blocking agent which inhibits LHR-mediated binding.

25. A method of claim 24, wherein the blocking agent
15 selectively binds LHR.

26. A method of claim 25, wherein the blocking agent comprises a carbohydrate moiety which selectively binds LHR, the carbohydrate having at least one of the following a sialic 20 acid residue, fucose residue, or a sulfate group.

27. A method of claim 25, wherein the blocking agent is an immunoglobulin.

28. A method of claim 24, wherein the blocking agent
selectively binds a recognition determinant on myelin.

29. A method of claim 28, wherein the blocking agent
is an isolated LHR.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92.05836

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) A61K 37/00; GO1N 33/53
 US CL 514/23, 903

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/23, 903

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,294,818 (McMichael et al) 13 October 1981, (entire document).	1-29
A	US, A, 4,839,276 (Adolfsen et al) 13 June 1989, (entire document).	1-29
A	US, A, 4,752,563 (Kortright et al) June 1988, (entire document).	1-29
A	US, A, 4,818,686 (Kortright et al) 04 April 1989 (entire document)	1-29
A	US, A, 4,935,343 (Allison et al) 19 June 1990, (entire document).	1-29
A	US, A, 4,948,726 (Longoria) 14 August 1990, (entire document).	1-29
A,P	US, A, 5,036,102 (Bachynsky et al) 30 July 1991, (entire document).	1-29
A	US, A, 4,994,466 (Sherman et al) 19 February 1991, (entire document).	1-29
A	US, A, 4,618,601 (Chazot et al) 21 October 1986, (entire document).	1-29
A,P	US, A, 5,089,479 (Krivan et al) 18 February 1992, (entire document).	1-29

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* "A" document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* "E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
* "O" document referring to an oral disclosure, use, exhibition or other means		
* "P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

21 AUGUST 1992

Date of mailing of the international search report

21 AUG 1992

Name and mailing address of the ISA/
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

Authorized officer

RONALD W. GRIFFIN

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/05836

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A, 0,153,875 (Spencer) 04 September 1985 (entire document).	1-29
A	EP, A, 0,184,040 (Rigdon) 11 June 1986, (entire document).	1-29
A	<u>J. Immunol.</u> , Volume 140, ISSUED 15 May 1988, D.O. Willenborg et al. "INHIBITION OF ALLERGIC ENCEPHALOMYELITIS IN RATS BY TREATMENT WITH SULFATED POLYSACCHARIDES", pages 3401-3405.	1-29
A	<u>The FASEB Journal</u> , Volume 3, ISSUED June 1989, D.O. Willenborg et al. "Phosphosugars are potent inhibitors of central nervous system inflammation", pages 1968-1971.	1-29
A	<u>ANALYTICAL BIOCHEMISTRY</u> , Volume 130, ISSUED 1983, C.G. Glabe et al. "Preparation and Properties of Fluorescent Polysaccharides", pages 287-294.	1-29
A	<u>The Journal of Cell Biology</u> , Volume 96, ISSUED March 1983, L.M. Stoolman et al. "Possible Role for Cell-surface Carbohydrate-binding Molecules in Lymphocyte Recirculation", pages 722-729.	1-29
A	<u>NATURE</u> , Volume 304, ISSUED 07 July 1983, W. M. Gallatin et al., "A cell-surface molecule involved in organ-specific homing of lymphocytes", pages 30-34.	1-29
A	<u>The Journal of Cell Biology</u> , Volume 99, ISSUED October 1984, L.M. Stoolman et al. "Phosphomannosyl Receptors May Participate in the Adhesive Interaction between Lymphocytes and High Endothelial Venules", pages 1535-1540.	1-29
A	<u>The Journal of Cell Biology</u> , Volume 104, ISSUED March 1987, T.A. Yednock et al. "Receptors Involved in Lymphocyte Homing: Relationship between a Carbohydrate- binding Receptor and the MEL-14 Antigen", pages 725-731.	1-29
A	<u>Cell</u> , Volume 56, ISSUED 24 March 1989, L.A. Lasky et al., "Cloning of a Lymphocyte Homing Receptor Reveals a Lectin Domain", pages 1045-1055.	1-29
A	<u>SCIENCE</u> , Volume 243, ISSUED 03 March 1989, M.H. Siegelman et al., "Mouse Lymph Node Homing Receptor cDNA Clone Encodes a Glycoprotein Revealing Tandem Interaction Domains", pages 1165-1172.	1-29
A	<u>Proc. Natl. Acad. Sci. USA</u> , Volume 87, ISSUED March 1990, T.K. Kishimoto et al. "Identification of a human peripheral lymph node homing receptor: A rapidly down- regulated adhesion molecule", pages 2244-2248.	1-29
A	<u>The Journal of Cell Biology</u> , Volume 111, ISSUED September 1990, Y. Imai et al., "Direct Demonstration of the Lectin Activity of gp90MEL, a Lymphocyte Homing Receptor", pages 1225-1232.	1-29
A	<u>NATURE</u> , Volume 346, ISSUED 02 August 1990, T.A. Springer, "Adhesion receptors of the immune system", pages 425-434.	1-29
A	<u>NATURE</u> , Volume 349, ISSUED 21 February 1991, O. Spertini et al. "Regulation of leukocyte migration by activation of the leukocyte adhesion molecule-1 (LAM-1) selectin", pages 691-694.	1-29
A	<u>Science</u> , Volume 245, ISSUED 15 September 1989, T.K. Kishimoto et al., "Neutrophil Mac-1 and MEL-14 Adhesion Proteins Inversely Regulated by Chemotactic Factors". pages 1238-1241.	1-29
A	<u>NATURE</u> , Volume 349, ISSUED 10 January 1991, S.R. Watson et al. "Neutrophil influx into an inflammatory site by a soluble homing receptor-igG chimaera", pages 164- 167.	1-29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/05836

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<u>The Journal of Cell Biology</u> , Volume 110, ISSUED June 1990, S.R. Watson et al, "A Homing Receptor-IgG Chimera as a probe for Adhesive Ligands of Lymph Node High Endothelial Venules", pages 2221-2239.	1-29
A	<u>TRANSPLANTATION</u> , Volume 48, No. 5, ISSUED November 1989, M.A. Jutila et al, "INFLAMMATION-INDUCED ENDOTHELIAL CELL ADHESION TO LYMPHOCYTES, NEUTROPHILS, AND MONOCYTES", pages 727-731.	1-29
A	<u>The Journal of Immunology</u> , Volume 143, No. 10, ISSUED 15 November 1989, M.A. Jutila et al, "FUNCTION AND REGULATION OF THE NEUTROPHIL MEL-14 ANTIGEN IN VIVO: COMPARISON WITH LFA-1 AND MAC-1", pages 3318-3324.	1-29
A	<u>The Journal of Immunology</u> , Volume 122, No. 5, ISSUED May 1979, B.J. Kutterer et al, "SELECTIVE ADHERENCE OF LYMPHOCYTES TO MYELINATED AREAS OF RAT BRAIN", pages 1666-1671.	1-29
A	<u>The Journal of Cell Biology</u> , Volume 104, ISSUED March 1987, T.A. Yednock et al, "Phosphomannosyl-derived Beads Detect a Receptor Involved in Lymphocyte Homing", pages 713-723.	1-29
A	Immunology Today, Vol 10, No. 9, issued 1989, Coombe et al, "Lymphocyte homing receptors...adhesion", pages 289-291.	1-29